

BrdU pulse labeling

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An abbreviated version of this protocol was published in eLIFE in Dec 2019

Primary cilia deficiency in neural crest cells models anterior segment dysgenesis in mouse

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Detailed protocol

BrdU preparation and administration

- Prepare stock solution of BrdU 10mg/ml in sterile PBS. BrdU powder is in the small -20°C fridge. Store the aliquots at -20°C in darkness for a maximum time of 3-4 months. Once you thaw the aliquoted vial keep it at 4°C for 5 days max.
- Inject intraperitoneally 150-200 ul per mouse of BrdU 10mg/ml. Ideally, you want to inject 50ug/bodyweight.
- Wait 2 hours and then sac the animal

BrdU staining on cryosections

- 1/2 head fixed in 4% PFA 15 min at 4C
 - Wash 3X15' in PBS
 - Incubate 1/2 heads in 30% sucrose in PBS O/N at 4C
 - OCT embedding
 - Fix slides in 4% PFA for 10 min at RT
 - Wash in 1X PBS 2x10' at RT shaking. In the meantime warm up 2N HCl at 37°C
 - Place slides in 2N HCl and incubate for 25' at 37°C
 - Wash in 1X PBS for 5' shaking
 - Blocking step: PBS + 0.1%Triton+ 2% NDS+ 1% BSA
 - Abs: rat anti-BrdU (Abcam ab6326) diluted between 1:100 and 1:400 in PBS +0.1% triton +1% BSA, O/N
- Day after:
- Wash 0.1%Triton +1% BSA 2x10'
 - Abs: donkey anti rat-FITC 1:200 for 1 h at RT
 - Wash 0.1%Triton +1% BSA 2x10'
 - Vectashield +Dapi (do not use Hoechst 33342)

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Iomini, C. and Portal, C. (2020). BrdU pulse labeling. Bio-protocol Preprint. bio-protocol.org/prep194.
2. Portal, C., Rompolas, P., Lwigale, P. and Iomini, C.(2019). Primary cilia deficiency in neural crest cells models anterior segment dysgenesis in mouse. eLIFE. DOI: [10.7554/eLife.52423](https://doi.org/10.7554/eLife.52423)

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